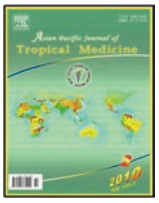
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading

Effect of *Phyllanthus niruri.* Linn on burn wound in rats

Tara Shanbhag, Arul Amuthan*, Smita Shenoy, Sudhakar

Department of Pharmacology, Kasturba Medical College, Manipal, Karnataka, India

ARTICLE INFO

Article history:
Received 28 August 2009
Received in revised form 30 September 2009
Accepted 12 November 2009
Available online 20 February 2010

Keywords:
Phyllanthus niruri
Burn wound
Dexamethasone suppressed wound healing
Wound contraction
Epithelialisation period

ABSTRACT

Objective: To evaluate the effect of ethanolic extract of *Phyllanthus niruri*.Linn (Euphorbiaceae) on experimentally induced burn wound model in rats and to evaluate whether it reverses the wound healing in steroid suppressed rats. **Methods:** Two models including burn wound model and dexamethasone suppressed burn wound model were used in the study. The formulations of ethanolic extract of *Phyllanthus niruri* were prepared in gum acacia at 8% and in ointment base at 10% and were administered orally (400 mg/kg) and externally respectively. The parameters studied were the wound contraction and the period of epithelialisation. **Results**: In burn wound model, oral and topical administration of *Phyllanthus niruri* did not show any significant effects in wound contraction and period of epithelialisation when compared to control. In dexamethasone suppressed burn wound model, wound contraction rate was increased significantly by topical ($P < 0.001$) and oral ($P < 0.001$) administrations of *Phyllanthus niruri* by about 47.57% and 26.16% respectively. Topical administration has shown significant ($P < 0.05$) enhancement of wound contraction than oral dosage form. Dexamethasone depressed epithelialisation period was reversed significantly by topical ($P < 0.0001$) and oral ($P < 0.001$) administrations of *Phyllanthus niruri* by about 32.5% and 21.3% respectively. **Conclusions:** Both topical and oral administrations of ethanolic extract of *Phyllanthus niruri* are found to reverse dexamethasone suppressed burn wound healing.

1. Introduction

The management of burn injury in patients having poor healing mechanism is one of the greatest challenges in clinical practice. Complications may deepen the depth of injury or convert a partial-thickness burn to a full-thickness injury [1]. Poor wound healing is observed either due to certain catabolic diseases like chronic diabetes mellitus or due to long term glucocorticoid therapy for various ailments. Thus it is essential to develop drugs which enhance wound healing mechanisms. Several drugs obtained from herbs are known to hasten the healing of wounds. The wound healing efficacy of certain traditionally used herbs remains unexplored.

The commonly available herb *Phyllanthus niruri.*Linn (Euphorbiaceae) is used traditionally in India for its medicinal properties. This herb was reported to possess anti-inflammatory[2], antifungal, antiviral, antibacterial[3], antioxidant, hepatoprotective[4], hypoglycemic[5], hypotensive, analgesic activities [6], inhibitory effect on renal stone formation etc[7]. The plant contain acidic arabinogalactan[8], diterpenes[9], alkaloids, flavonoids, lignans, tannins, terpenes [10], hypophyllanthin, phyllanthin[11] etc. In the present study, we evaluated the effect of *Phyllanthus niruri* on burn wound and steroid suppressed burn wound healing by oral and topical administration.

2. Materials and methods

2.1. Experimental animals

Male albino wistar rats weighing 250-300 g were used. The animals were caged individually after making burn wound till completion of wound healing. In each group six animals were used. The experimental protocol was approved by Institutional Animal Ethics Committee and animals were maintained under standard conditions in animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2. Drugs and chemicals

Ketamine injection was obtained from Neon Laboratories

*Corresponding author: Dr. Arul Amuthan, Post graduate, Department of Pharmacology, Kasturba Medical College, Manipal, Karnataka, India-576104
Tel: 08202922365
E-mail: dramuthanmd@yahoo.co.in

Limited (Mumbai, India), Silver sulphadiazine was obtained from Kasturba Hospital Pharmacy (Manipal), dexamethasone was obtained from Zydus Alidac (Ahmedabad) and Gum acacia was obtained from Nice Chemicals Ltd (Cochin). Dried *Phyllanthus niruri* was purchased from local market and authenticated by Department of Pharmacognosy, MCOPS, Manipal. Voucher specimen was kept in the department.

2.3. Preparation of extract

A finely macerated dried whole plant, weighing 500 grams, was soaked in ethanol measuring 3 liters approximately, in a 5 liter round flask for about 24 hours. After 24 hours, extraction was started by reflux condensation at 60-80 ℃. The process was done for 3 hours. Then it was cooled and the ethanol was drained using a muslin cloth into a conical flask. Then, fresh absolute ethanol was put into the round bottom flask containing the plant material. The same procedure of reflux condensation was repeated twice with 3 liters of ethanol each time. The ethanol recovered from these three batches, that is, the extract measuring approximately nine liters was concentrated under reduced pressure by distillation till it acquired a syrupy consistency. Finally, the extract from three batches was mixed in a big china dish and evaporated to dryness on a water bath, later; it was weighed to find out the yield.

2.4. Burn wound

Partial thickness burn wounds were made on overnight-fasted animals under ketamine (50 mg/kg, IM) anesthesia by pouring hot molten wax (2 g) at 80 ℃ [12]. The wax was poured on the shaven back of the animal through a cylinder of 300 mm^2 circular opening. The wax was allowed to remain on the skin for 8 minutes by which time it got solidified. This was day 0.

In dexamethasone suppressed burn model, dexamethasone was administered from day 0 (0.17 mg/kg, I.P) and was continued on subsequent days till the day of eschar falling [13].

2.5. Selection of doses and treatment period

The dose selected was 400 mg/kg body weight of rat after doing acute toxicity study. For oral administration, suspension of ethanol extract (8%) was prepared using 2% gum acacia. For topical application, ointment of ethanol extract (10%) was prepared using simple ointment base. The drugs were administered once daily from day 1 and continued till the day of falling of eschar in both models.

2.6. Study design

Burn wound model and dexamethasone suppressed burn wound model were used to asses the wound healing property of *Phyllanthus niruri* in rats. Four and three groups of animals were used for burn model and dexamethasone suppressed burn model respectively. There were six animals in each group. The drug treatment was as follows:

For Burn Wound Model:
Group 1: Gum acacia (2%) 2 mL by oral (Control);
Group 2: Silver sulphadiazine (0.5g of 1%) cream, topical;
Group 3: *Phyllanthus niruri* extract (400 mg/kg) oral;
Group 4: *Phyllanthus niruri* extract (10%) topical;
For dexamethasone suppressed burn wound model:
Group 5: Dexamethasone (0.17 mg/kg, I.P) (DEXA group);
Group 6: Dexamethasone (0.17 mg/kg, I.P) and *Phyllanthus niruri* extract (10%) topical;
Group 7: Dexamethasone (0.17 mg/kg, I.P) and *Phyllanthus niruri* extract (400 mg/kg) oral.

2.7. Evaluation of burn wound healing activity

Burn wound model was utilized to evaluate the rate of wound contraction and the time required to full epithelialisation of the wound.

2.7.1. Wound contraction rate

Wound area was measured by tracing the wound on a transparent butter paper on every alternate day of post-wounding. The tracing was then transferred to 1 mm^2 graph sheets, from which the wound area was evaluated. The evaluated surface was then employed to calculate the percentage of wound contraction, taking the initial size of wound 300 mm^2 as 100% by using the following equation:

$$\%\ \text{of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

For the analysis of serial measurements of wound contraction on each subject on different days, the Area Under Curve (AUC) [14] of each subject was calculated. The AUC was calculated by adding the areas under the graph between each pair of consecutive observations obtained from percentage of wound contraction. If we have measurements y_1 and y_2 at times t_1 and t_2, then the AUC between those two times is the product of the time difference and the average of the two measurements. Thus we get $(t_2-t_1).(y_1+y_2)/2$. If we have n+1 measurements y_i at times t_i (i=0, 1, 2..... n), then the AUC is calculated as:

$$AUC = 1/2 \sum_{i=0}^{n-1} (t_{i+1} - t_i)(y_i + y_{i+1})$$

Thus percentage of wound contraction was converted in to Area Under Curve. In burn wound model, the % enhancement of wound contraction rate was calculated by taking AUC of control group as 100% and by using the following formula:

$$\%\ \text{Enhancement of wound contraction rate} = \frac{\text{AUC of Control group} - \text{AUC of } \textit{Phyllanthus niruri} \text{ group}}{\text{AUC of Control group}} \times 100$$

In steroid suppressed burn model, taking the AUC of DEXA group as 100%, the percentage enhancement of wound contraction rate was calculated.

2.7.2. Period of epithelialisation

Falling of the eschar leaving no raw wound behind was taken as end point of complete epithelialisation and the days required for this was taken as period of epithelialisation. Percentage decrease in period of epithelialisation was calculated by the same formula as in % enhancement of wound contraction rate.

2.8. Statistical analysis

Results were expressed as mean ± SEM. The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Posthoc Test viz Tukey Alpha (0.05). The results were considered statistically significant at $P<0.05$.

3. Results

3.1. Wound contraction rate

In burn wound model, oral and topical administrations of *Phyllanthus niruri* did not show any significant in wound contraction compared to control and silver sulphadiazine treated groups (Table1).

In dexamethasone suppressed burn wound model, wound contraction rate was increased significantly by topical ($P<0.001$) and oral ($P<0.001$) administration of *Phyllanthus niruri* by about 47.57 % and 26.16% respectively. Topical administration has shown significant ($P<0.05$) enhancement of wound contraction than oral administration. (Table 1, Figure 1).

3.2 Period of epithelialisation

In burn wound model, oral and topical administrations of *Phyllanthus niruri* did not reduce the period of epithelialisation compared to control and Silver sulphadiazine treated groups (Table 2).

In dexamethasone suppressed burn wound model, topical ($P<0.0001$) and oral ($P<0.001$) administration of *Phyllanthus niruri* significantly reversed dexamethasone depressed epithelialisation period by about 32.5% and 21.3% respectively (Table 2, Figure 1).

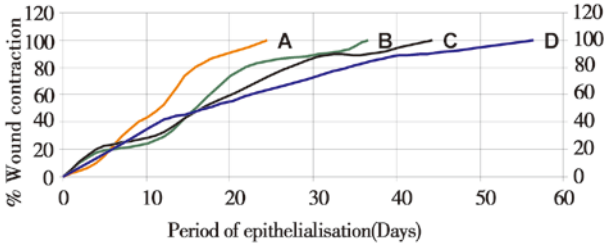


Figure 1. Steroid suppressed burn wound model: Effect of *Phyllanthus niruri* in reversing the delaying of wound contraction rate and epithelialisation period.
From burn wound model: A = Control group;
From dexamethasone suppressed burn model: D=Dexamethasone induced delaying in wound healing, B= Dexamethasone + *Phyllanthus niruri* topical, C = Dexamethasone + *Phyllanthus niruri* oral.

Table 1
Burn wound model--effect of Phyllanthus niruri on wound contraction rate.

Groups	Drugs	Dose & Route	% Wound contraction (Mean ± SEM)	% Enhancement of wound contraction
Group 1	Gum acacia	2 mL, Oral	284.95 ± 139.14	–
Group 2	Silver Sulphadiazine	1%, Topical	1 175.62 ± 65.60	8.56
Group 3	*Phyllanthus niruri*	400 mg/kg, Oral	1 267.48 ± 165.77	1.36
Group 4	*Phyllanthus niruri*	10%, Topical	1 573.35 ± 225.59	–22.45
Group 5	Dexamethasone	0.17 mg/kg, I.p	3 617.44 ± 208.05	–
Group 6	Dexamethasone + *Phyllanthus niruri*	0.17 mg/kg, I.p+400 mg/kg, Oral	1 896.75±194.55[a,c]	47.57
Group 7	Dexamethasone + *Phyllanthus niruri*	0.17 mg/kg, I.p+10%, Topical	2 671.14 ±131.44[b]	26.16

n=Number of animals in each group = 6; compared to dexamethasone group; compared to dexamethasone group, [a]$P<0.0001$, [b]$P<0.001$ compared to dexamethasone + *Phyllanthus niruri* topical group, ;[c]$P<0.05$. [ANOVA was followed by Posthoc Test viz Tukey Alpha (0.05)].% contraction values are converted into Area Under Curve.

Table 2
Burn wound model-effect of *Phyllanthus niruri* on period of epithelialisation.

Groups	Drugs	Dose & Route	Period of epithelialisation (Mean ± SEM)	% enhancement of epithelialisation period
Group 1	Gum acacia	2 mL, Oral	24.50± 2.34	–
Group 2	Silver sulphadiazine	1%, Topical	21.00 ± 1.15	14.29
Group 3	*Phyllanthus niruri*	400 mg/kg, Oral	21.50 ± 1.56	12.25
Group 4	*Phyllanthus niruri*	10%, Topical	26.16 ± 2.83	-6.77
Group 5	Dexamethasone	0.17 mg/kg, I.p	56.33 ± 1.89	–
Group 6	Dexamethasone + *Phyllanthus niruri*	0.17 mg/kg, I.p + 400 mg/kg, Oral	36.50 ± 1.45[d]	32.5
Group 7	Dexamethasone + *Phyllanthus niruri*	0.17 mg/kg, I.p +10%, Topical	44.33 ± 1.52[e]	21.3

n=Number of animals in each group = 6; compared to dexamethasone group, [d]*P*<0.0001; compared to dexamethasone group, [e]*P* <0.001; [ANOVA was followed by Posthoc Test viz Tukey Alpha (0.05)].

4. Discussion

Initial inflammatory phase, proliferation & migration of epithelial cells, granulation tissue formation and wound contraction are the main phases of burn wound repair mechanism. Neovascularisation takes place under the influence of vascular endothelial growth factor (VEGF). Platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), basic fibroblast growth factor (bFGF) and cytokines are the factors which are associated with cellular proliferation [15]. In burn wound model, *Phyllanthus niruri* did not significantly alter healing of burn wound as compared to control.

Dexamethasone delays burn wound healing by reducing transforming growth factor- β (TGF- β), collagen & fibroblast deposition, neovascularisation and epithelial migration in wounds[16-18]. Hence, decreased wound contraction rate and delaying in period of epithelialisation were observed in dexamethasone suppressed burn wound model. In dexamethasone suppressed burn wound model, topical or oral administration of *Phyllanthus niruri* significantly increased wound contraction rate and enhanced the period of epithelialisation.

Since *Phyllanthus niruri* enhances steroid suppressed wound healing significantly, it could be used as a supportive therapy to treat chronic wounds in catabolic diseases like diabetes mellitus etc. Though mechanism of action of Phyllanthus niruri responsible for the reversal of steroid suppression in healing was not studied, yet it could be the objective of further studies.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1]Yurt RW. Burns and inhalation injury. In: Norton JA, Barie PS, Bollinger RR, editors. *Surgery – basic science and clinical evidence*. New York, USA: Springer Science; 2008, p. 447.
[2]Thyagarajan SP, Jayaram S, Gopalakrishnan V, Hari R, Jeyakumar P, Sripathi MS. Herbal medicines for liver diseases in India. *J Gasrtoenterol Hepatol* 2002;**3**:370.
[3]Farouk A, Bashir AK, Salih AKM. Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity (I). *Fitotherapia* 1983;**54**(1):3.
[4]Sabir SM, Rocha JBT. Water-extractable phytochemicals from *Phyllanthus niruri* exhibit distinct *in vitro* antioxidant and *in vivo* hepatoprotective activity against paracetamol-induced liver damage in mice. *Food Chemistry* 2008;**111**:845.
[5]Upal KM, Malaya G, Yerra R. Antihyperglycemic effect and antioxidant potential of *Phyllanthus niruri* in streptozotocine induced diabetic rats. *European Bulletin of Drug Research* 2005;**13**:15.
[6]Santos ARS, Filho VC, Yunes RA, Calixto JB. Further studies on the antinociceptive action of the hydroalcoholic extract from plants of the genus *Phyllanthus*. *J Pharm Pharmacol* 1995;**47**:66.
[7]Frietas AM, Schor M, Boim MA. The effect of *Phyllanthus niruri* on urinary inhibitors of calcium oxalate crystalisation and other factors associated with renal stone formation. *BJU Int* 2002;**89**:829.
[8]Mellinger CG, Carbonero ER, Noleto GR, Carbonero ER, Oliveira MBM, Gorin PAJ, et al. Chemical and biological properties of an arabinogalactan from *Phyllanthus niruri*. *J Nat Prod* 2005;**68**:1479.
[9]Hossain MA, Salehuddin SM, Diterpenes from the leaves of *Phyllanthus niruri*. *Indian Journal of Natural Products* 2006; **22**:18.
[10]Li Xiang-rong. Chemical components and bioactivities of *Phyllanthus niruri* L. *Tianran Chanwu Yanjiu Yu Kaifa* 2007;**19**:890.
[11]Van Dau, N. Ha TTT. Chemical composition of *Phyllanthus* niruri L., Euphorbiaceae. *Tap Chi Duoc Hoc -Saigon Then Hanoi*, 2007;**47**:15-18.
[12]Bairy KL, Somayaji SW, Rao CM. An experimental model to produce partial thickness burn wound. *Ind J Exp Biol* 1997; **35**:70.
[13]Shanbhag T, Shenoy S, Rao MC. Wound healing profile of Tinospora cordifolia. *Indian Drugs* 2005;**42** (4) : 217 -221.
[14]Matthews JNS, Altman DG, Campbell MJ, Royston P, Analysis of serial measurements in medical research. *Br Med J* 1990;**300**:230.
[15]Mohan H. *Textbook of pathology*. New Delhi: Jaypee Brothers Medical Publishers Ltd;2005, p.173-76.
[16]Ehrlich HP, Hunt TK, Effect of cortisone and vitamin A on wound healing. *Ann Surg* 1968;**167**:324.
[17]Ehrlich HP, Traver H, Hunt TK. The effect of vitamin C and glucocorticoids upon inflammation and collagen synthesis. *Ann Surg* 1973;**177**:222.
[18]Wicke C, Halliday B, Allen D, Roche NS, Scheuenstuhl H, Spencer MM, et al. Effect of steroids and retinoids on wound healing. *Arch Surg* 2000;**135**:1265.